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REMARKS

By the present amendment, the specification has been amended to correct apparent typographical errors and/or to improve its presentation. Independent claims 1, 2, 4-7 and 15 have been amended to obviate the examiner's objections thereto and/or to further clarify the concepts of the present invention. Entry of these amendments is respectfully requested.

In the Office Action, the restriction requirement between claims 11-13 of Group I drawn to a kit for measuring a component of a sample and claims 1-12 and 14-23 of Group II drawn to a method for separating a complex substance using an electrode was reiterated. As required, the provisional election of the claims of Group II is hereby affirmed.

Claim 15 was objected to as containing an apparent typographical error. The correction of this informality has been accomplished by the amendments herein.

It was suggested that the specification be reviewed as for possible minor errors and requested correction of any errors located. As mentioned above, the specification has been amended to correct apparent typographical errors and/or to improve its presentation.

Claims 1-10 were rejected under 35 USC § 102(e) as being anticipated by the Regnier et al patent. Additionally, claims 14-23 were rejected under 35 USC § 103(a) as being unpatentable over the same Regnier et al patent. Briefly, in making the former rejection, it was asserted that the Regnier et al patent teaches a method which forms a complex of a molecule to be measured, separates the specific complex of the molecule by use of a dielectrophoretic field, and then detects the separated molecule complex to give a quantitative measurement.

In making the latter rejection, basically the previous assertions regarding the Regnier et al patent were relied upon and it was further asserted that the patent teaches the application of a high electrical potential to ensure adequate mixing. Although it was acknowledged that the specific minimum value for the applied voltage as claimed was not taught by the patent, it was asserted that the use of such a voltage would be obvious to one of ordinary skill in the art to determine through routine experimentation. Reconsideration of these rejections in view of the above claim amendments and the following comments is respectfully requested.

It is submitted that the Regnier et al patent does not teach or suggest the invention as defined by the amended claims. More particularly, the Regnier et al patent does not teach, among other things, a method which utilizes

dielectrophoresis. Briefly, dielectrophoresis relies upon the motion imparted on uncharged particles as a result of polarization induced by a non-uniform electric field. In contrast, the method as disclosed by the cited Regnier et al patent is directed to electrophoresis which does not involve the application of a non-uniform electric field. The differences between electrophoresis (electric migration) and dielectrophoresis (dielectric migration) are explained in detail in the following.

### 1. Electric migration procedure

The electric migration procedure is based on the principle that when an object with a positive or negative charge is placed in an electric field, the object is given a force to move toward the electrode having the charge opposite to the object. In this regard, attention is directed to the attached Fig. 1A and 1B. The object with a positive or negative charge is referred to as "charged object," and the force is referred to as "electric migration force," and such phenomenon is referred to as "electric migration." The electric field used in this procedure is generally a homogeneous or uniform one.

The electric migration force ( $F_E$ ) is shown by the following formula:

$$F_E = qE$$

wherein "q" represents a charge provided on an object, and "E" represents an electric field.

It is apparent that, in the electric migration, the force given to the object is essentially affected by the given charge ( $q$ ) of the object. In case of  $q=0$ , that is in the condition of neutral, where the object has no charge, or in case of the object being placed on the isoelectric point, the given force becomes  $F_E=0$  so that the object cannot move as shown in Fig. 1C. Therefore, it is essential that the object to be transferred has to be charged in case of the electric migration procedure.

In order to separate several objects based on the principle of the electric migration, the difference of the charges given to the objects, respectively, may make separation so as to be transferred to the specific portions, respectively. Other than the given charges, such condition as size and structure may slightly affect the migration.

## 2. Dielectric migration procedure

The dielectric migration procedure is based on the principle that when an object is placed in an heterogeneous or nonuniform field, a positive or negative polarization is generated in the object to be a force to move as is explained on, for example, page 3, lines 1-8 of the specification. The force is referred to as "dielectric migration force."

The object in the electric field is induced to have a positive polarization charge, "+q," on the downstream side (negative side) of the field, and a negative polarization charge, "-q," on the upstream side (positive side) of the field. This generates a force "+qE," which draws the object portion with the charge, "+q," to the downstream side of the electric field, and a force, "-qE," which draws the object portion with charge, "-q," to the upstream side of the electric field as is illustrated in the attached Fig. 2A.

In the heterogeneous electric field, the larger force would be generated in the stronger electric field, resulting in that the object tends to transfer itself to the stronger electric field. In this regard, attention is directed to Fig. 2B, as well as page 13, line 21 to page 14, line 15, and Fig. 1 of the subject specification. Thus, it is essential that the object should be placed in a heterogeneous electric field in case of the dielectric migration procedure.

It is noted that the object is not always drawn toward the stronger electric field. Whether the object is drawn to a stronger or weaker electric field depends on the angular frequency of the electric field, and the dielectric constant and electric conductivity of the object.

The dielectric migration force, "F<sub>d</sub>," is shown by the following formula.

$$F_d = 2\pi a^3 \epsilon_m \operatorname{Re}[K^*(\omega)] \Delta (E^2)$$

wherein "π" represents the circle ratio; and "a" represents a radius of the object; and "ε<sub>m</sub>" represents the dielectric constant of the object; and [K\*(ω)] represents a value calculated from the angular frequency of the applied voltage, and the dielectric constant and electric conductivity of the object and medium such as solution; and, "E" represents electric field. "\*" shows the number is a complex number, Re[] shows that the number is an actual number, and "Δ" shows a gradient.

It is apparent that the dielectric migration force is significantly affected by K\*(ω), that depends on the angular frequency of the applied voltage and the dielectric constant and electric conductivity of the object and medium, as well as 2πr<sup>3</sup>, that is, the size of the object as explained on page 26, lines 10-14 of the subject specification. It should be noted that the charge of the object, "q," does not affect the force.

Therefore, the dielectric migration procedure operates on any objects, regardless of the charging of the objects. Further, in view of the above, it is to

be recognized that, with the dielectric migration procedure, it is possible to move a neutral object which is impossible by the electric migration procedure as is illustrated in Fig. 2B.

From the above explanation, it is apparent that the dielectric migration procedure is completely different from the electric migration and thus the presently claimed methods which utilize the dielectric migration procedure patentably distinguish over the electric migration procedure as disclosed by the Regnier et al patent. To further emphasize this distinction over the cited patent to Regnier et al, independent claims 1, 2 and 4-7 have been amended to further describe the dielectrophoresis procedure which utilizes a non-uniform electric field. Particularly, these claims include, as independent claims 14-17 now recite, the feature of the use of a non-uniform electric field.

For the reasons stated above, withdrawal of the rejections under 35 U.S.C. § 102(a) and § 103(a) and allowance of claims 1-10 and 14-23 over the cited Regnier et al patent are respectfully requested.

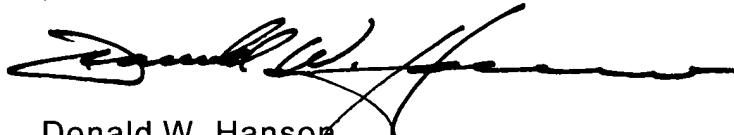
In view of the foregoing, it is submitted that the subject application is now in condition for allowance and early notice to that effect is earnestly solicited.

Serial Number: 09/670,399

In the event this paper is not timely filed, the undersigned hereby petitions for an appropriate extension of time. The fee for this extension may be charged to Deposit Account No. 01-2340, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,

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Marked Up Version of Amendments to Specification and Claims

IN THE SPECIFICATION:

Amend the specification as follows:

(Page 3, line 9 to page 4, line 4): These separation methods are presently believed to be the most suitable separation method in m-TAS from the following points: (1) a rapid separation can be expected at a low applied voltage without requiring a high voltage as in capillary electrophoresis, since an electric field and its gradient can be increased to an extreme ~~extend~~ extent if micromachined electrodes are employed, because the degree of dielectrophoretic forces depends on the size and dielectric properties of substances (particles) and is proportional to the electric field gradient; (2) an increase in temperature due to applying the electric field can be minimized, and a high electric field can be formed, since a strong electric field area is localized at a significantly small region; (3) as the dielectrophoretic force is a force proportional to the electric field gradient, the force is understood as independent on the polarity of the applied voltage, and thus works under an AC electric field in a similar way to a D.C. electric field, and therefore if a high frequency A.C is employed, an electrode reaction (electrolytic reaction) in an aqueous solution can be suppressed, so that the electrodes themselves can be integrated in the channel (sample flow path); (4) improvement in a detection sensitivity can be expected, since there is no restriction to a chamber volume of the detection component as in capillary electrophoresis, and the like.

(Page 5, lines 12-19): However, reports on separation methods with conventional dielectrophoretic forces as described above are limited to separating particles having a low solubility in a solution, relative to DNAs and proteins, such as various cells and latex particles, or otherwise only capturing a single (one kind of) DNA or protein, and any report has not been presented yet on separation of respective molecules from solutions in which are dissolved two or more kinds of biological component molecules, in particular, such as for example DNAs and proteins.

(Page 6, lines 11-14): It is also an object of the present invention to provide a method for separating each other two or more kinds of molecules dissolved in a solution , by using dielectrophoretic forces , such separation having so far been impossible.

(Page 9, line 22 to page 11, line 9): ~~more~~ More specifically, the present invention relates to a method for detecting a molecule to be measured in a sample, which comprises reacting a liquid sample, in which a "molecule to be measured" is dissolved, and a solution, in which a "substance specifically binding to the molecule to be measured" is dissolved, to obtain a solution in which a complex substance of the "molecule to be measured" and the "substance specifically binding to the molecule to be measured", and the "substance specifically binding to the molecule to be measured" which is not

involved in the reaction are dissolved, placing the solution under a nonuniform electric field having an electric field strength of 500 KV/m or higher, the field being formed by electrodes which have a structure capable of forming a horizontally and vertically ununiform electric field, separating the complex substance from the "substance specifically binding to the molecule to be measured" which is not involved in the reaction, and measuring the "substance specifically binding to the molecule to be measured" in the complex substance, or the the "substance specifically binding to the molecule to be measured" which is not involved in the reaction; and a method for measuring a substance to be measured in a sample comprising, reacting a liquid sample containing a "molecule to be measured", a "molecule to be measured labeled by a labeling substance", and a "substance specifically binding to the molecule to be measured" to obtain a solution containing a complex substance of the "molecule to be measured labeled by a labeling substance" and the "substance specifically binding to the molecule to be measured", a complex substance of the "molecule to be measured" and the "substance specifically binding to the molecule to be measured", and the "molecule to be measured labeled by a labeling substance" which is not involved in the reaction, placing the obtained solution under a nonuniform electric field having an electric field strength of 500 KV/m or higher, the field being formed by electrodes which have a structure capable of forming a horizontally and vertically ununiform electric field, separating the complex substance of the "molecule to be measured labeled by a labeling substance" and

the "substance specifically binding to the molecule to be measured" from the "molecule to be measured labeled by a labeling substance" which is not involved in forming the complex, and then measuring the "molecule to be measured labeled by a labeling substance" in the complex substance or the "molecule to be measured labeled by a labeling substance which is not involved in forming the complex substance to determine the amount of the molecule to be measured in the sample based on the results.

(Page 14, line 16 to page 17, line 1): Samples to which the present invention can be applied include samples derived from living body such as body fluids including serum, plasma, cerebrospinal fluid, synovial fluid, lymph, etc., excreta including urine, feces, etc., and treated materials thereof. Treated materials include diluted solutions of these samples derived from a living body in water, buffers, or the like, or those reconstituted by appropriately dissolving or suspending molecules as ~~describes~~ described below from these body-derived samples in water, buffers, or the like. Samples to which the present invention is applied also include those containing the above described molecules which are chemically synthesized .

(Page 17, line 6 to page 18, line 9): A "substances capable of changing dielectrophoretic properties" in the present invention (also referred

to a separation improving substance) includes a substance which, by binding to a specific molecule (molecule to be measured) to form a complex with the specific molecule, causes differences in behavior to dielectrophoretic operation between the specific molecule and the other co-existing substances (molecules not to be measured, for example, one or more kinds of substances which are not involved in the formation of the complex): for example 1) a substance which can cause a result that any one of both is captured on the dielectrophoresis electrode and the others are not ~~capture~~ captured, and more specifically, a substance which can provide changes in the movement speed of the specific molecule and the other co-existing substances, for example, in the case of employing a so-called dielectrophoretic chromatography apparatus (Field Flow Fractionation apparatus) in which separation is carried out as described below with the interaction between dielectrophoretic forces caused by the molecules in the electric field and the molecular movement, and more preferably, a substance by which any one of these can be captured on the electrode and the others can be passed through on the dielectrophoresis electrode without being captured on the electrode; or 2) a substance which can cause a result that any one of both receives negative dielectrophoretic forces and the others receive positive dielectrophoretic forces, and more specifically, a substance which, for example, can allow only the specific molecule to gather at a particular position on the dielectrophoretic electrode, and more preferably,

a substance which can allow any one of these to gather at a strong electric field strength region on the dielectrophoresis electrode by positive dielectrophoretic forces and the others to gather at a weak electric field strength region on the dielectrophoresis electrode by negative dielectrophoretic forces; or the like.

(Page 19, lines 1 to 13): A "substance binding to a specific molecule" which can be used in the present invention may not be limited in particular and includes a substance which, from a "specific molecule" in a sample, can form a complex substance of the "specific molecule", a "substance binding to the specific molecule" and a "specific substance capable of changing dielectrophoretic properties", and does not substantially form a complex substance of "molecules other than the specific molecule", the "substance binding to the specific molecule" and the "specific substance capable of changing dielectrophoretic properties". In short, so long as the substance does not form the latter complex substance of the above-mentioned three substances , it can be used ~~for~~ for this purpose even if it binds to molecules other than the "specific molecule". Actually, a "substance specifically binding to the specific molecule" is preferably used.

(Page 35, line 6 to page 36, line 8): In the case where the separation is carried out by method (2) of Separation Method-2 described above, the

specific molecule or the other molecule can be collected respectively, under conditions where the separation improving substance and the specific molecule bound to the separation improving substance have positive dielectrophoretic forces and the molecules other than the specific molecule have negative dielectrophoretic forces, by collecting at first a mobile phase which contains the molecules other than the specific molecule having negative dielectrophoretic forces and moving without being captured at a particular position on the electrode, and after that, collecting a washed solution which contains the specific molecule by moving the specific molecule having positive dielectrophoretic forces which is captured at a particular position on the electrode during applying the electric field by ceasing from applying the electric field and washing the electrode with an appropriate buffer usually employed in the art, water, or the like. Alternatively, the specific molecule or the other ~~other~~ molecule can be collected respectively , under conditions where the molecules other than the specific molecule have positive dielectrophoretic forces and the separation improving substance and the specific molecule bound to the separation improving substance have negative dielectrophoretic forces, by collecting at first a mobile phase which contains the specific molecule having negative dielectrophoretic forces and moving without being captured at a particular position on the electrode, and after that, collecting a washed solution which contains the molecules other than the specific molecule by moving the

molecules having positive dielectrophoretic forces and having been captured at a particular position on the electrode during applying the electric field by ceasing from applying the electric field and washing the electrode with an appropriate buffer usually employed in the art, water, or the like.

(Page 41, lines 9-14): When ~~solutions~~ a solution as described previously has a high conductivity, Joule heat generates by the current flowing in the solution as the voltage is applied, resulting in possibilities of boiling the solution. Therefore, it is preferable that the solutions are used with appropriate adjustment such that the conductivity is usually in the range of not more than 10 mS/cm, preferably not more than 200  $\mu$ S/cm.

(Page 68, lines 17-21): In the above-mentioned methods, the nucleotide probe and buffers can be selected appropriately according to methods known per se. Method for preparing a nucleotide probe and unknown genes denatured to the single strand, annealing conditions, and the like can be performed according to methods known per se per se.

(Page 75, line 17 to page 76, line 1): Until now, it is impossible to separate a complex of biotin and a fluorescein-labeled anti-biotin antibody from an unreacted fluorescein-labeled anti-biotin antibody by dielectrophoretic chromatography and the detection of a complex with biotin

has not been achieved, because there is no difference in dielectrophoresis separation between the complex and the unreacted antibody to a sufficient extent extent. The above-mentioned results indicate that applications of a separation improving substance can permit to detect quantitatively by dielectrophoretic chromatography, molecules which have not been detected until now.

(Page 77, lines 14-16): After the antigen-antibody reaction was completed, the reaction solutions were diluted 100 times with distillated distillied water, and the resultants were subject to the dielectrophoretic separation.

(Page 80, lines 4-6): After the antigen-antibody reaction was completed, the reaction solutions were diluted to 100 times with distillated distillied water to and the resultants were subjected to dielectrophoresis.

(Page 80, lines 10-14): The results are shown in Figure 11. It can be found from Figure 11 that a good quantitativeness is obtained within the range of the presence of AFP. From this finding, it is understood that, if serum is used as samples, components in the serum do not affect dielectrophoresis to a great extent extent, and the detection of a protein to be measured in serum can be achieved.

(Page 82, lines 5-9): In SSC buffer, 0.05 % (w/v) of the 2kb λDNA probe immobilized latex beads was added to the labeled singled-stranded λDNA and T7 DNA to the final concentration of 20μg/ml, and hybridization was carried out at 68 °C for 18 hours. The sample solution after the hybridization reaction was diluted 100 times with ~~distilled~~ distilled water, and subjected to dielectrophoresis.

(Page 90, line 17 to page 91, line 11): In this Example, taking account of the event where all the λ DNA are captured and the oligonucleotide is not captured at all, the capture ratio is equal to the percentage of the fluorescence amount derived from the λ DNA occupied in the fluorescence amount of a whole sample. That is, Sample 1 (a sample having a mixing ratio of 0:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 100 %, Sample 2 (a sample having a mixing ratio of 1:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of  $1/(1+1) = 50\%$ , Sample 3 (a sample having a mixing ratio of 5:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of  $1/(1+5) = 16.7\%$ , and Sample 4 (a sample having a mixing ratio ratio of 1:0 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 0 %.

(Page 94-95 - Table 4, column 3, row 1, line 3):

Table 4

Sample No.	Concentration Biotin-labeled λ DNA	Concentration Fluorescein-labeled <del>anti-biotin</del> anti-biotin antibody	Concentration unlabeled λ DNA
1	0μg/ml	21μg/ml	10μg/ml
2	2.5μg/ml	21μg/ml	7.5μg/ml
3	5μg/ml	21μg/ml	5μg/ml
4	10μg/ml	21μg/ml	0μg/ml

IN THE CLAIMS:

Amend the claims as follows:

1. (Amended) A method for separating a complex substance of a "specific molecule" in a sample and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule" from molecules other than the "specific molecule" in the sample, comprising

forming the complex substance of the "specific molecule" and the "substance capable of changing dielectrophoretic properties of the specific molecule", and

applying the resulting reaction mixture containing the complex substance to dielectrophoresis using a nonuniform electric field, and

separating the complex substance from molecules other than the "specific molecule".

2. (Amended) A method for determining an amount of a component in a sample, comprising

forming a complex substance of a "specific molecule" in a sample and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule",

applying the resulting reaction mixture containing the complex substance to dielectrophoresis using a nonuniform electric field,

separating the complex substance from molecules other than the "specific molecule",

measuring the "specific molecule" in the separated complex substance or a molecule other than the "specific molecule" in the sample, and

determining an amount of the component in the sample on the basis of the measurement result.

4. (Amended) A method for separating a complex substance of a "specific molecule" in a sample, a "substance binding to the specific molecule" and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule" from the "substance binding to

the specific molecule" which is not involved in forming the complex substance, comprising

contacting the sample containing the "specific molecule" with the "substance binding to the specific molecule", and the "substance capable of changing dielectrophoretic properties of the specific molecule" to form the complex substance, and

applying the resulting reaction mixture containing the complex substance to dielectrophoresis using a nonuniform electric field, and

separating the complex substance from the "substance binding to the specific molecule" which is not involved in forming the complex substance.

5. (Amended) A method for detecting a "specific molecule" in a sample, comprising

contacting a sample containing a "specific molecule" with a "substance binding to the specific molecule", and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule" to form a complex substance of the "specific molecule", the "substance binding to the specific molecule", and the "substance capable of changing dielectrophoretic properties of the specific molecule",

applying the resulting reaction mixture containing the complex substance to dielectrophoresis using a nonuniform electric field,

separating the complex substance from the "substance binding to the specific molecule" which is not involved in forming the complex substance,

measuring the "substance binding to the specific molecule" in the separated complex substance ,and

detecting the presence or absence of the "specific molecule" in the sample on the basis of the measurement result.

6. (Amended) A method for determining an amount of a component in a sample, comprising

contacting a sample containing a "specific molecule" with a "substance binding to the specific molecule" and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule" to form a complex substance of the "specific molecule" and the "substance capable of changing dielectrophoretic properties of the specific molecule",

applying the resulting reaction mixture containing the complex substance to dielectrophoresis using a nonuniform electric field,

separating the complex substance from the "substance binding to the specific molecule" which is not involved in forming the complex substance,

measuring the "specific molecule" or the "substance binding to the specific molecule in the separated complex substance or the "substance binding to the specific molecule" which is not involved in forming the complex substance, and

determining an amount of the component in the sample on the basis of the measurement result.

7. (Amended) A method for determining an amount of a component in a sample, comprising

contacting a sample containing a "specific molecule" with a "specific molecule labeled by a labeling substance", and a "substance capable of changing dielectrophoretic properties of the specific molecule" which binds to the "specific molecule" to form a labeled complex substance of the "specific molecule labeled by the labeling substance" and the "substance capable of changing dielectrophoretic properties of the specific molecule",

applying the resulting reaction mixture containing the labeled complex substance to dielectrophoresis using a nonuniform electric field,

separating the labeled complex substance from the "specific molecule labeled by the labeling substance" which is not involved in forming the complex substance,

measuring the "specific molecule labeled by the labeling substance" in the separated labeled complex substance or the "specific molecule labeled by the labeling substance" which is not involved in forming the complex substance, and

determining an amount of the component in the sample on the basis of the measurement result.

15. (Amended) A method for detecting a molecule to be measured in a sample, which ~~comprisis~~ comprises

reacting a liquid sample, in which a "molecule to be measured" is dissolved, and a solution, in which a "substance specifically binding to the molecule to be measured" is dissolved, to obtain a solution in which a complex substance of the "molecule to be measured" and the "substance specifically binding to the molecule to be measured", and the "substance specifically binding to the molecule to be measured" which is not involved in the reaction are dissolved,

placing the solution under a nonuniform electric field having an electric field strength of 500 KV/m or higher, the field being formed by electrodes which have a structure capable of forming a horizontally and vertically ununiform electric field,

separating the complex substance from the "substance specifically binding to the molecule to be measured" which is not involved in the reaction,

measuring the "substance specifically binding to the molecule to be measured" in the complex substance, and

detecting the presence or absence of the "molecule to be measured" in the sample on the basis of the measurement result.